ABSTRACT

The complexes of the oxidative phosphorylation (OXPHOS) system in the inner mitochondrial membrane are organised into structural and functional super-assemblies, so-called supercomplexes. This type of organisation enables substrate channelling and hence improves the overall OXPHOS efficiency. ATP synthase associates into dimers and higher oligomers. Within the supercomplex of ATP synthasome, it interacts with ADP/ATP translocase (ANT), which exchanges synthesised ATP for cytosolic ADP, and inorganic phosphate carrier (PiC), which imports phosphate into the mitochondrial matrix. The existence of this supercomplex is generally accepted. Experimental evidence is however still lacking.

In this thesis, structural interactions between ATP synthase, ANT and PiC were studied in detail. In addition, the interdependence of their expression was examined either under physiological conditions in rat tissues or using model cell lines with ATP synthase deficiencies of different origin. Specifically, they included mutations in the nuclear genes *ATP5E* and *TMEM70* that code for subunit ε and the ancillary factor of ATP synthase biogenesis TMEM70, respectively, and a microdeletion at the interface of genes *MT-ATP6* and *MT-COX3* that impairs the mitochondrial translation of both subunit *a* of ATP synthase and subunit Cox3 of cytochrome *c* oxidase.

Functional and structural characterisation of the cell lines with ATP synthase defects revealed that nuclear mutations in the genes *TMEM70* and *ATP5E* (the first reported mutation in a nuclear gene coding for a structural subunit of ATP synthase) lead to a reduced content of fully functional ATP synthase. In contrast, a mutation in *MT-ATP6* is accompanied by a normal amount of incomplete ATP synthase that is non-functional due to the lack of subunit *a*. In this case, the pathological phenotype manifests itself above 90 % heteroplasmy of mutated mtDNA. At all the studied defects, a compensatory up-regulation of ANT and PiC was found, likely due to an adaptive mechanism at the post-transcriptional level. Under physiological conditions, however, the expression of ATP synthase, ANT and PiC appears to be co-regulated at the level of transcription.

Although structural analyses revealed the existence of ATP synthasome in rat heart mitochondria, the majority of ATP synthase, ANT and PiC were found as separate entities. The functional significance of ATP synthasome therefore still remains controversial. The analyses also detected an association of ATP synthase with succinate dehydrogenase that had been previously reported as the so-called mitochondrial ATP-sensitive K^+ channel.